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Boreal peatland ecosystems are well known as an important sink of atmospheric mercury (Hg) as well as a source of highly toxic methylmercury (MeHg) to downstream ecosystems. Therefore, a thorough understanding on the complex Hg cycling in these northern ecosystems is important. Stable Hg isotopes were evaluated as a tool to investigate Hg cycling in an upland-peatland ecosystem in northern Minnesota. Stable Hg isotopes undergo both mass-dependent fractionation (MDF; denoted by δ^{202}Hg) and mass-independent fractionation (MIF; denoted by Δ^{199}Hg) during biotic, abiotic, and photochemical reactions, and are diagnostic of sources and transformations of Hg in the environment. In this study, we examined the variability of stable Hg isotopes in exported waters in a relatively well characterized small peatland-upland watershed (S2) in Marcell Experimental Forest in northern Minnesota, U.S.A. Exported water samples at the watershed outlet were collected biweekly when the stream flowed in both 2014 and 2015. Additionally, we collected surface and subsurface runoff from upland soils, porewater from the lagg zones, bog cores, upland soil, foliage and litter, for determining total-Hg, MeHg, and Hg stable isotope compositions. Data on exported waters over time (2014-2015) show a large range of MDF (δ^{202}Hg range from -2.1 to -1.3 ‰; n=21) but only a small range of MIF (Δ^{199}Hg range from -0.35 to -0.10 ‰; n=21), with Δ^{199}Hg resembling the isotopic values of Hg in bog and foliage samples. Samples with more positive δ^{202}Hg in the exported waters were collected during the spring snow melt event, and these δ^{202}Hg

values are similar to the values of surface and subsurface runoff from the upland (i.e., -1.4 to -1.2 ‰; n=4). Our results suggest that the temporal variation of Hg isotopes in exported waters is large in this small upland-peatland watershed, which was driven by the hydrology in the watershed. The differences in MDF values of Hg may allow us to distinguish the two sources of Hg in the exported waters as derived from upland forest *vs.* peatland bog. Therefore, stable Hg isotopes could be potentially used in other peatland and wetland ecosystems for understanding the complex Hg cycling in the environment.

INVESTIGATING SOURCES OF SEASONAL VARIATION IN MERCURY EXPORTED FROM AN UPLAND-PEATLAND ECOSYSTEM IN NORTHERN MINNESOTA USING MERCURY STABLE ISOTOPES

by

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CHAPTER I

INTRODUCTION

1.1 Background

Mercury (Hg) is a naturally occurring trace element. There are both natural and anthropogenic sources of Hg in the environment. Although the use of Hg has been phased out in many commercial products, human populations continue to be exposed to increasing levels of Hg through various media (UNEP, 2013). Mercury is a neurotoxin that deteriorates the nervous system affecting behavior, reproductive success, and motor function (Bose-O'Reilly et al. 2010, Kolka et al. 2011). Many studies focused on aquatic ecosystems due to the bioaccumulation and biomagnification of methylmercury (MeHg), a highly toxic form of Hg, in aquatic food webs. Consumption of fish represents the primary exposure to Hg in both human and wildlife populations (UNEP 2013). Mercury is the leading cause of fish consumption advisories throughout the United States (EPA 2011). The increasing number of populations affected by Hg exposure has brought increased international awareness to the issue of Hg contamination.

Mercury naturally cycles through our environment, but increased atmospheric loading has been attributed to the increased anthropogenic (Mason & Sheu 2002). The majority of Hg is stored in reservoirs in earth's crust. However, Hg in its elemental form Hg is a gas (i.e., $Hg^0_{(g)}$) naturally emitted by volcanos, and ocean hydrothermal vents.

Compared to pre-industrial levels, $Hg^0_{(g)}$ concentrations have increased about three folds in the atmosphere due primarily to anthropogenic contributions (UNEP 2013).

The largest anthropogenic contributors of Hg to the atmosphere are artisanal gold mining and coal burning, contributing 37% and 24% of annual emissions, respectively (UNEP 2013). Due to its relative inertness, $Hg^0_{(g)}$ can travel in the atmosphere up to 1 year (Butler et al. 2007). Elemental Hg ($Hg^0_{(g)}$) mixes in the atmosphere before being globally distributed and deposited, creating a non-point source of Hg pollution for the majority of ecosystems.

Atmospheric Hg enters ecosystems through either wet or dry deposition (Branfireun et al. 2005). $Hg^0_{(g)}$ is about 95% of the atmospheric pool of Hg (Zhang et al. 2009, Lindberg et al. 2007, Gustin et al. 2008), with the rest being the oxidized forms of Hg. For example, $Hg^0_{(g)}$ is oxidized by ozone and other free radicals to form Hg^{2+}, a more reactive and soluble species of Hg that can readily dissolve in precipitation and deposit onto the landscape, as wet deposition. A larger contribution of deposition occurs as dry deposition via stomatal uptake of Hg^0 and subsequently as leaf litter fall (Zhang et al. 2012, Hintelmann et al. 2002). Between 25-60% of atmospheric Hg that is deposited onto terrestrial landscape would eventually enter nearby water bodies (Swain et al. 1992). Depending on environmental compartments, 1-10% of deposited Hg can be microbially transformed into MeHg in anoxic environment such as wetland soil and lake sediment (Selevendiran et al. 2008). The remaining Hg can either be retained in the soil or

sediment through binging to natural organic matter, or be re-emitted back to the atmosphere via photoreduction (Engstrom & Swain 1997).

Peatlands are globally distributed and account for ~5% of land area (Figure 2). Due to very slow decomposition rates in peatlands, Hg bound to organic matter is accumulated over time. Unlike other elements, (e.g., iron, aluminum, and manganese), Hg does not mix vertically in redox condition, which preserves the temporal record of Hg deposition (Benoit et al.1994). Ombrotrophic bogs are hydrologically isolated from groundwater and surface water sources. Therefore, Hg entering into these ecosystems is primarily due to atmospheric deposition (Kolka et al. 2000, Zillioux et al. 1993). Peat accumulation rates and Hg concentrations at varying depths display historical trends of global atmospheric loading.

Peatlands play an important role in Hg cycling because they are regarded as a sink of atmospheric Hg, but peatland can also be a source of Hg and MeHg to downstream ecosystems. Peat is resulted from the accumulation of *Sphagnum* moss, a non-vascular plant, which acquires nutrients for growth from the atmosphere. These plants have sorption values for elements that are an order of magnitude higher than other aquatic plants (Gignac et al. 1987). As peat degrades, there would result in high levels of humic and fulvic acid that could exist as dissolved organic matter (DOM) and/or particulate organic matter (POM) in the peatland waters. DOM and POM are rich in reduced thiol groups that have a high binding affinity to Hg and MeHg (Verry et al. 1999). Mercury bound to the DOM/POM-rich waters are exported from peatlands and mobilized in surface waters (Mierle et al. 1991). With its extensive reducing conditions,

peatlands are also hot spots for the production of MeHg by various groups of anaerobic bacteria capable of methylating Hg, such as sulfate reducing, iron reducing, and methanogenic bacteria (Yu et al. 2010; Gilmour et al. 2013). Bacterial communities colonize *Sphagnum* moss and have even been suggested to provide a syntropic relationship (Yu et al. 2010, Opelt et al. 2007). Besides microbial methylation, atmospheric sources could also be a source of MeHg to the peatland ecosystems such as rainfall and snowfall (Hall et al. 2005; Mitchell et al. 2008).

Mercury cycling has been studied for 3-4 decades, but due to the complex nature and the uncertainty in estimating contributions there are many knowledge gaps in our understanding of Hg cycling in natural ecosystems. Advancements in multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) has allowed for increased precision and accuracy, with proper sample introduction approach and mass-bias corrections high-precision stable Hg isotope measurements have been shown to be feasible (Hintelmann et al. 2012, Blum et al. 2014). These advancements have allowed high-precision isotopic composition measurement of Hg in many natural samples and it is now the exclusive method used to quantify stable Hg isotope compositions in various environmental samples (Hintelmann et al. 2012, Blum et al. 2014).

During the isotopic measurements, the abundance of the six to seven stable Hg isotopes (^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{204}Hg) in environmental samples was measured and compared to an isotopic standard (SRM NIST 3133) (Blum & Bergquist 2007). Changes of the composition of isotope ratios among samples relative to the standard are regarded as isotopic fractionation. Many abiotic and biotic processes can

fractionate Hg isotopes. Bergquist & Blum (2007) demonstrated that Hg stable isotopes can undergo two types of isotopic fractionation in nature: mass-dependent fractionation (MDF) and mass-independent fractionation (MIF). MDF occurs in all kinetic reactions that do not go to completion, as lighter isotopes react faster, therefore, the reactant becomes enriched with heavier isotopes compared to the product. MDF is calculated using the equation below:

$$\delta^{xxx}Hg\ (‰) = \{[(^{xxx}Hg/^{198}Hg)_{sample}/\ (^{xxx}Hg/^{198}Hg)_{NIST3133}]\text{-}1\}*1000$$

In this equation xxx represents the mass number of each stable Hg isotope larger than ^{198}Hg, in this study, we use δ^{202}Hg to denote MDF in all samples.

Interestingly, MIF is caused by photochemical reactions but large-magnitude of MIF only occurs for the two odd-mass Hg isotopes, ^{199}Hg and ^{201}Hg. MIF has only been known to occur naturally with Hg binding to oxygen and sulfur ligands associated with DOM (Bergquist & Blum 2009). MIF is caused by the magnetic volume effect, which is due to the mass to charge ratio of the odd-mass isotope causes a difference in the reaction rates compared to even-mass Hg stable isotopes. The difference in reaction is symbolized as cap delta (Δ) and is calculated using the equation below:

$$\Delta^{xxx}Hg = \delta^{xxx}Hg - (\beta * \delta^{202}Hg)$$

In this equation xxx represents the mass number of ^{199}Hg or ^{201}Hg. Beta (β) in this equation represents a scaling constant for a theoretical kinetic MDF reaction. Depending on which isotope is analyzed (^{199}Hg, ^{200}Hg, ^{201}Hg, or ^{204}Hg) a different constant would be

applied, i.e., 0.2520, 0.5024, 0.7520, 1.493, respectively. In this study, we use Δ^{199}Hg to denote MIF in all samples.

In this study, we aimed to investigate the sources and cycling of Hg in an upland-peatland ecosystem in northern Minnesota by using stable Hg isotopes. Concurrent water table measurements and water chemistry analyses provided supporting data on how different events contribute to Hg exported from the ecosystem. We proposed to determine if the sources of Hg to the study ecosystem change seasonally. We aimed to identify sources of Hg contributions being exported from the ecosystem using stable Hg isotope compositions and mixing model analysis.

1.2 Site Selection

A relatively remote peatland in Itasca County, northern Minnesota, USA, was examined in this study (Figure 1). Marcell Experimental Forest (MEF) has six experimental watersheds monitored by the USDA Forest Service. MEF was one of the nation's first National Atmospheric Deposition Program (NADP) stations; monitoring atmospheric deposition of chemicals since 1977 (Kolka et al. 2011). MEF also hosts the nation's first Mercury Deposition Network (MDN) station, established in 1978 (Kolka et al. 2011).

Hydrology and water chemistry have been continuously recorded in MEF since 1977, whereas atmospheric Hg deposition has been recorded and analyzed by MDN in this location since 1993. These data are available for interannual comparisons. Watershed S2 has served as a control site for the experimental watersheds in MEF. Watershed S2 is

relatively small in size of only 9.7 ha; 3.2 ha of which is a bog dominated by *Sphagnum* moss and black spruce, and a 6.5 ha forested upland area dominated by trembling aspen and paper birch. It has been shown that Hg export increase with decreasing watershed size as larger watershed areas would dilute Hg concentrations by precipitation transported out of the ecosystem (Kolka et al. 1999). The small size of watershed S2 allows for greater event response and a measurable sensitivity to any disturbance including snowmelt and rainfall events. Upland soil of the S2 watershed is a sandy loam, rich in minerals. The bog soils are primarily organic matter accumulation ranging from 3-9 meters at the center of the bog (Kolka et al. 2001). Watershed S2 is isolated from groundwater and surface waters, called ombrotrophic, so that rainfall and snowfall represent the only water sources imported into the bog. Surface waters that exceed the regional water table level are exported through an outlet stream. The outlet stream typically flows from March/April to December, and is otherwise frozen. Stream flow may also cease during summer droughts. The pH of the exported water tends to be acidic at 3.9±0.2 due to the *Sphagnum* moss releasing hydrogen ions as it takes up other cations for growth (Mitsch & Gosselink 1993). Watershed S2 is shallow, allowing continuous mixing, keeping waters oxygenated, and saturated all year.

Watershed S2 is outfitted with a concrete V-notch weir that represents the watershed outlet and also serves the location of our sample collection for stream waters (or exported waters). Surface and subsurface runoff from the upland forest are collected from the northern slope of watershed S2. Surface waters flow though galvanized sheet metal funnels into polyvinyl chloride (PVC) piping that is collected and measured in a

holding tank. Subsurface waters flow through a welled trench that runs parallel with the A horizon soil into PVC piping that is collected and measured in a holding tank (Figure 2).

Previously, a mass balance approach was used to investigate sources and cycling of Hg and MeHg in watershed S2 (Kolka et al. 2001, Mitchell et al. 2008a). These studies have provided evidence that the bog and the lagg zone of the S2 watershed are sinks for atmospheric Hg. However, during high flow events the upland and lagg become a source that can contribute disproportionally to the amount of Hg exported from the watershed (Kolka et al. 2001). Watershed S2 is nutrient poor, which limits bacterial production of MeHg production as previously shown in a controlled experiment (Mitchell et al. 2008b). Using total Hg and MeHg concentration data it has been shown that snowmelt, in only a twelve-day period, contributes 26-39% of annual export of total Hg and 22-23% of annual export of MeHg from watershed S2 (Mitchell et al. 2008a). These findings implied that the source of Hg in the exported waters may vary over time in response to different hydrological events. For the first time, this study would investigate if the source of Hg changes seasonally and if the source of the variation can be accounted for by stable Hg isotope compositions in the exported waters.

1.3 Water Chemistry Analysis

Cation concentrations were collected throughout the study by USDA Forest Service and they reflect values similar to atmospheric levels recorded in the past. This ecosystem is not affected by Midwest industrial emissions (Verry 1983). It has been

shown that in oxygenated conditions Hg forms complexes with the reduced thiol groups of DOM (Miller et al. 2007). To demonstrate the association of Hg to organic matter, levels of total organic carbon (TOC) or dissolved organic carbon (DOC) are often correlated with total Hg and MeHg levels in natural waters (Brigham et al. 2009). Correlations between TOC/DOC and Hg are strongest during high flow events, and decreased in times of low flows. During drought bog waters become anoxic, creating conditions more favorable for microbial methylation of Hg.

1.4 Seasonal Variation of Stable Hg Isotope Ratios in the Export Waters of Watershed S2

It is predicted that there could be some variations in stable Hg isotope compositions in exported waters between seasons due to changes in environmental inputs. Snowmelt in spring represents about 1/4 of the estimated annual Hg import to S2 watershed (Mitchell et al., 2008a). In the spring, it is expected that peatland water stable Hg isotopes would be most similar to atmospheric Hg due to the accumulation of Hg^{2+} from snowfall. The major contributing source of Hg during the year to the watershed comes from the bog itself, as bog soil and/or vegetation act as a sink for atmospheric Hg (Kolka et al. 2001). In the summer months, when there are not as many rain events and the water table of watershed S2 declines, it is expected that stable Hg isotope signatures would resemble those of Hg stored in vegetation such as leaf litter or peat moss. Pre-industrially deposited Hg may also contribute as a source to surface waters during drought when Hg in deeper horizon of bog may release to the waters. Therefore, we

expected that stable isotope Hg signatures of the exported waters of watershed S2 may change over time reflecting multiple sources of Hg throughout the year.

1.5 Determining the Sources of Variation in Hg Exported from Watershed S2

It is expected that each environmental component collected could have a unique and traceable stable Hg isotope signature reflecting different sources of Hg deposited or transformations of Hg in the environment or within the watershed. Possible sources include atmospheric Hg as dry or wet deposition, or preexisting Hg stored in the watershed. We expected that Hg deposited in different depths of soil and peat may have different Hg stable isotope signatures based on whether the Hg was deposited by natural sources (e.g., pre-industrial) or with more anthropogenic contributions (e.g., post-industrial). The isotopic differences may be large enough to distinguish preindustrial deposits *vs.* contemporary sources of Hg reflected by the exported waters of watershed S2.

CHAPTER II

METHODS

2.1 Sample Collection

USDA Forest Service employees stationed in Grand Rapids, MN, sampled MEF watershed S2 exported waters for measuring water chemistry regularly in both 2014 and 2015. USDA Forest Service employees also collected watershed S2 exported waters from the V-notch weir at the watershed outlet biweekly, as well as surface and subsurface runoff (less frequent) when available after major hydrological events (e.g. snowmelt and rainfall). All water sample collection used ultraclean trace metal protocol to minimize inadvertent contamination of samples (EPA, 1996). Grab sample of watershed S2 exported waters was collected into acid-cleaned 2 L FEP bottle (Nalgene) previously preserved with 1% HCl. Each FEP bottle was rinsed with stream water three times before the sample was collected. After collection, the FEP bottles were double bagged, and stored in a cooler on ice. Samples were then shipped to UNCG overnight.

2.2 Water Chemistry Analysis

Discharge was measured at the V-notch weir and was continuously recorded onto a strip chart which was then digitalized to liters per second. In addition, a 100 mL of water sample were collected by USDA Forest Service employees for analyzing TOC and cation concentration in the USDA lab at Grand Rapids, MN.

2.3 Method Comparison

Exported water samples from watershed S2 appear brownish due to the extensive DOM present resulted from the degradation of *Sphagnum* moss (e.g., TOC values ranged from 7.5 to 116 mg C/L in our study period). The reduced thiol group in DOM has a very high binding affinity with Hg (Ravichandran 2004), making it difficult to separate Hg from the stream water samples for analyzing stable Hg isotope ratios. Traditional methods outlined by EPA protocol 1631 revision E use an oxidizing agent, bromine monochloride (BrCl) for digesting aqueous samples prior to analyzing for total Hg. This method works well for water samples with low levels of TOC or DOC as precipitation (Sherman et al. 2012). Using BrCl for digestion on OM-rich peatland waters, previous studies have shown recoveries of Hg ranging from 70 to 90% (Gu et al. 2011, Wang et al. 2015).

An alternative method to separate Hg from waters with complicated matrices such as wastewater influents and effluents would involve the use of an acidic mixture of permanganate and persulfate (i.e., HNO_3, H_2SO_4, $KMnO_4$ and $K_2S_2O_8$) at elevated temperature (95^oC). Using this method, it has been reported that 90-105 % of Hg was consistently recovered from various wastewater samples (Balogh et al.1996). High recoveries of sample Hg are necessary in order to preserve the original isotopic composition of Hg in the sample and to avoid artificial isotopic fractionation (mainly MDF) during sample processing. Breakdown of OM can also be achieved by ultraviolet (UV) radiation and can also allow for an efficient separation of Hg from OM in water samples, but this approach cannot be used in our study because UV radiation can

artificially fractionate stable Hg isotopes as demonstrated in a recent study (Mead et al. 2013). However, a UV-treated water sample could be used as a reference for comparing digestion recovery of Hg in water samples as a way to validate the efficiency of different digestion approaches for water samples with high OM content. In order to compare the recoveries of Hg of two different approaches on peatland waters, a 2 L sample of watershed S2 exported waters was collected and shipped to UNCG. Upon arrival, six aliquots each of 80 mL were transferred from the sample into acid washed 125 mL acid0-cleaned Teflon bottles. The sample was split into two treatments: one was digested using an acidic mixture of permanganate and persulfate according to Balogh et al. (1996) [i.e., 20 HNO_3:1 H_2SO_4:5 $KMnO_4$ (5% w/v): 2.5 $K_2S_2O_8$ (5% w/v)] at 95^oC for 6 to 8 hours. This solution was allowed to cool before being completely neutralized with hydroxylamine (NH_2OH). Another one was treated by using EPA protocol 245.7. The sample was oxidized with 5% (BrCl) and placed in an oven at 60^oC overnight, then (NH_2OH) was added to destroy free halogens plus an additional UV treatment at the University of Michigan. The UV treatment in addition to EPA protocol is assumed to completely break down OM and release all Hg in the sample.

Mercury from the digested water samples was analyzed by cold vapor atomic fluorescence spectroscopy spectrophotometer (CVAFS, Brooks Rand Instruments). Each treatment were run in replicate and averaged in order to determine the total Hg concentrations recovered from each digestion. Besides, the digested samples were spiked with a known amount of Hg standard and then run on CVAFS to demonstrate if there are any “undigested” binding sites in the aqueous solution.

2.4 Seasonal Variation of Hg Stable Isotopes in the Export Waters of an Upland-Peatland Ecosystem

Stable Hg isotopes from the exported waters of watershed S2 were analyzed in both 2014 and 2015. Upon arrival at UNCG, the sample was acid digested utilizing the acidic mixture of permanganate and persulfate (Balogh et al. 1996). Afterward, digested samples were completely neutralized by hydroxylamine and two aliquots each of 40 mL were taken from the sample to measure total-Hg concentrations of the water samples and also used to compare recovery of Hg after the "purge and trap" procedure. Mercury separated from the sample were concentrated using a modified "purge and trap" based on previously published methods to concentrate Hg from 2 L of water sample into a ~6 g trap of 1 % $KMnO_4$ solution that would be used to run for stable Hg isotope ratios by MC-ICP-MS at the University of Michigan (Gratz et al. 2010, Sherman et al. 2012).

Here is a brief description of the purge and trap procedure to concentrate Hg for stable isotope analysis. One liter of digested water sample is placed in a clean 2 L borosilicate glass bottle with a Corning GL45 three-hole delivery cap, and attached to a Teflon tubing connected to a glass sparger in a glass trap with ~6 g of 1% $KMnO_4$ in 10% H_2SO_4 (i.e., sample trap for Hg), which is connected to an air cadet vacuum pressure pump. Besides, a peristaltic pump is used to continuously deliver 10% $SnCl_2$ at 1 mL/min to reduce $Hg^{2+}_{(aq)}$. Trace-metal grade sulfuric acid was added to the sample mixture in order to maintain an acidic pH throughout the purge and trap process. The vacuum created in the system introduces ambient air (passing through a 0.45-μm filter and gold trap to remove particulates and gaseous Hg) through the sample at a rate of about

40 L/min. The introduced air was used to direct any reduced $Hg^0_{(g)}$ from the water samples into the sample trap downstream (Figure 3). The vacuum pump would remain active for an additional 30 minutes after completing the delivery of 10% $SnCl_2$ to ensure all $Hg^0_{(g)}$ was purged from the sample into sample trap.

Once the samples were taken down, a small aliquot of 1% $KMnO_4$ would be collected in duplicate, and neutralized with hydroxylamine for analysis of total Hg by CVAFS (Figure 4) to determine the recovery of Hg following the purge and trap (Liang et al. 1993). A percent recovery was calculated for each purge and trap process (Gratz et al. 2010, Sherman et al. 2012). Samples with recoveries between 90-100% would be regarded as sufficiently high for analyzing stable Hg isotopes (or no significant MDF) and would be shipped to the University of Michigan's Biogeochemistry and Environmental Isotope Geochemistry Laboratory for stable Hg isotope analysis using cold-vapor multi-collector inductively coupled plasma mass spectrometry (CV-MC-ICP-MS, Nu Instruments) (Blum & Bergquist 2007).

2.5 Determining the Sources of Variation in Hg Exported from the S2 Watershed

Environmental components were collected in April 2015 from MEF watershed S2 including cores from the bog, lagg, and upland forest. Leaf litter, foliage, pine needles in bogs, and peat moss were also collected. Surface and subsurface runoff were collected by USDA Forest Service employees when available and shipped to UNCG on ice. Cores were collected with an acid washed Russian corer to a depth of 50 cm and cores were divided into 5 cm sections with a stainless steel knife and shipped to UNCG overnight on

ice. All water samples utilized methods in section 3.2 to separate Hg from the matrix and concentrate Hg for subsequent isotopic analysis. All solid samples including soil and vegetation were freeze-dried and homogenized using a SPEX sample prep ceramic mixer mill. Total Hg concentrations of solid samples were determined using acid digestion (HNO_3:H_2O_2, 4:1 v/v) in PFA digestion vessels (Savillex) at 80^{o}C overnight, aliquots were analyzed in duplicate by CVAFS for Hg content.

Quality control and quality assurance of all sample digestion and analyses were closely monitored by including a reagent blank and two standard reference materials (SRMs) including apple leaves (NIST 1515) and marine sediment (NRCC MESS-3), that were acid-digested and analyzed along with the samples. Analytical runs were checked throughout with two Hg standards, NIST 3133 and NIST 1641d. Total Hg concentrations acquired from the acid digestion was used to calculate percent recovery for processing solid samples for stable Hg isotope measurements.

To separate Hg from solid samples, a two-stage thermal combustion method was used (Figure 5). Homogenized samples were weighed and placed in ceramic boats, and placed in quartz combustion tube. The first furnace slowly heated up from room temperature to 750^{o}C over six hours in order to slowly release sample Hg in the form of $Hg^{0}_{(g)}$. The second furnace was held at 1000 ^{o}C with quartz wool for decomposing combustion by-products. High-purity oxygen (Hg-free) was used to assist combustion and also direct $Hg^{0}_{(g)}$ released from the sample into a ~24 g of 1% $KMnO_4$ sample trap (in 10% H_2SO_4). Heat tapes were wrapped around the inlet and outlet of the combustion tube and is maintained at 90-100^{o}C to prevent moisture condensation from forming on

the exposed end of the quartz tube that may interfere the transfer of $Hg^0_{(g)}$ to the sample trap.

Following combustion, the samples were taken down and the 1% $KMnO_4$ sample trap was neutralized by hydroxylamine, and aliquots were taken to measure Hg content (in duplicate) in order to calculate the combustion recovery of Hg. If the combustion recovery was between 90-100%, the sample solution was further concentrated into a 6 g trap of 1% $KMnO_4$ sample trap using the "purge and trap" method shown (Figure 6).

Samples were analyzed on CVAFS for total Hg concentrations in order to calculate the recovery of purge and trap. Samples with a total recovery between 90-100% compared to the original combusted samples were then sent to the University of Michigan for stable Hg isotope analysis by CV-MC-ICP-MS.

Stable Hg isotope ratios from water samples and environmental components were compared using an isotope mixing model (e.g. Phillips & Gregg 2003) to determine the relative contributions of different environmental components to Hg in exported waters in watershed S2 over sampling time, the equations are listed below.

$$X\Delta^{199}Hg_{\text{(source 1)}} + Y\Delta^{199}Hg_{\text{(source 2)}} + Z\Delta^{199}Hg_{\text{(source 3)}} = \Delta^{199}Hg \text{ sample}$$

$$X\delta^{202}Hg_{\text{(source 1)}} + Y\delta^{202}Hg_{\text{(source 2)}} + Z\delta^{202}Hg_{\text{(source 3)}} = \delta^{202}Hg \text{ sample}$$

$$X + Y + Z = 1$$

Using the equations listed above the proportion of three different sources were simultaneously calculated to determine the contribution of each source to the MDF and

the MIF in the mixture. These equations take into account the variability of both the mixture as well as the source.

CHAPTER III

RESULTS

3.1 Verification of Sample Digestion Method

Compared to the BrCl and UV treatment, our approach of using the acidic mixture of permanganate and persulfate recovered an average of 93% of Hg in exported waters from watershed S2 (Figure 7). Our approach of sample digestion is assumed to not artificially fractionate isotopic compositions of Hg in the samples, supporting that this digestion method could be used in this study to extract Hg from OM-rich peatland waters for stable Hg isotope analysis. The findings indicated that the binding sites on the organic matter in the peatland waters were mostly (if not all) broken down during sample digestion.

Additionally, digested sample spiked with a known amount of Hg standard (NIST 3133) and reanalyzed for Hg concentrations. Post spike analysis recovered between 96-98% from $KMnO_4/K_2S_2O_8$ digested sample *vs.* and 103-104% from the BrCl and UV treatment (Figure 8). This provides further evidence that the binding sites in the digested water samples are mostly destroyed, and sample Hg should be released upon the addition of $SnCl_2$ during purge and trap procedure to separate Hg for isotopic analysis.

3.2 Temporal Variation of Discharge, Total Hg, and MeHg in the Exported Waters

Discharge from watershed S2 varied over time and differed by years. In 2014, discharge ranged from 0 to 8.1 L/s with a maximum flow occurring from April through June. Flow remained low during the summer months starting from July and stopped flowing completely throughout August and November in 2014 (Figure 9A). In 2015, discharge ranged from 0 to 7.6 L/s with a maximum discharge in April and November (Figure 9B). Temporal variability for total Hg concentrations in exported waters were observed for both years and in general total Hg concentrations increased with discharge of the stream flow (Figure 9A and 9B). Discharge and total Hg concentrations were regressed using two different categories: non-event flow and event flow. A weak but positive relationship was noted for non-event flow (r^2=0.24) and total Hg concentrations during event flow (r^2= 0.79). Similarly, temporal variability for MeHg concentrations in exported waters were observed for both years and in general MeHg concentrations decreased with discharge of the stream flow (Figure 10A and 10B). However, weaker regression relationships were found between discharge and MeHg concentrations during non-event flow (r^2=0.22) and event flow (r^2=0.13).

Relatively, MeHg is only a small fraction of total Hg in the exported waters, ranging from 0.3 to 11% of total Hg as MeHg in samples collected in 2014 and from 0.1 to 7.9% of total Hg as MeHg in samples collected in 2015.

3.3 Temporal Variation of Stable Hg Isotope Ratios in the Exported Waters

Data on exported water over time show a large range of δ^{202}Hg (-2.1 to -1.3 ‰; n=13) (Figure 11). Samples with more positive δ^{202}Hg in the exported water were collected during the spring snowmelt event, and these δ^{202}Hg values are similar to the values of surface and subsurface runoff from the upland (i.e., -1.4 to -1.2 ‰; n=3). Stable Hg isotopes show the exported waters of watershed S2 with variation in MDF indicating multiple source contributions throughout the year. Variation falls within the reported stable Hg isotope values for soil and foliage (Biswas et al. 2008, Demers et al. 2013, Estrade et al. 2011, Tsui et al. 2012). The more positive MDF values may be influenced by an atmospheric source. The more negative MDF values may be influenced by vegetation. Exported waters show a small range of $\mathbf{\Delta}^{199}$Hg (-0.35 to -0.1 ‰; n=13) (Figure 12), with $\mathbf{\Delta}^{199}$Hg resembling the values of bog and foliage samples (Demers et al. 2013).

3.4 Determining the Sources of Variation in Hg Exported from Watershed S2

Environmental components collected from watershed S2, namely surface runoff, subsurface runoff, and lagg porewater had more positive MDF values than the bog cores (Figure 13A). In contrast, MIF values of Hg were relatively similar among all environmental samples collected from watershed S2 in both years (Figure 13B), therefore, MIF signature of Hg may be less helpful in distinguishing sources of Hg in the exported waters.

Literature values of stable Hg isotope ratios in atmospheric gas, precipitation (rainfall), vegetation and soil were compiled and presented in Figure 14. The individual data points can be found in Table 3 in Appendix. In order to determine the sources contributing to the variation in the MDF of Hg in the exported waters over time, a triple mixing model (Phillips & Gregg 2003) was applied. Variability of MDF values of Hg was seen in the exported waters in both 2014 and 2015.

Based on the isotopic values complied from the literature (Figure 14), exported waters from watershed S2 fall mostly within the ranges of soil and foliage (here grouped as "watershed") (Figure 15). Samples with more positive MIF values may be influenced by precipitation and samples with more positive MDF values could be influenced by precipitation and/or atmospheric Hg^0 (Figure 15). Therefore, the endmembers chosen for the triple mixing model were precipitation Hg, atmospheric Hg, and watershed Hg. The proportion of each contribution was calculated taking into consideration the variability of MDF and MIF simultaneously.

The mixing model results show that in the majority of samples watershed (soil and foliage) is the main source to Hg exported from watershed S2 in both years (Figure 16A and 16B). For a few samples in April 2014, we found that precipitation would contribute up to 25% of Hg in the exported waters (Figure 16A).

If we focus on the samples only within watershed S2 we collected from both 2014 and 2015, we found that there are large and significant differences of MDF values of Hg among different components: we found much higher MDF for upland sources compared to lower MDF for peatland sources (bog) (Figure 17). The hydrograph shows that high

flow events may influence the MDF values of Hg exported from watershed S2. In general, when the discharge is high Hg in the exported waters would have higher MDF values and look closer to MDF values of Hg from upland runoff (Figure 17). Lower values of MDF in the exported waters occur during low flow and these MDF values resemble the lower MDF values from the bog core (Figure 17). During both years of study, we found that Hg in the exported waters had MDF values between these two endmemners (i.e., upland and peatland), depending on the hydrology.

CHAPTER IV

DISCUSSION

Peatland waters (runoff, porewater and stream water) are well known to contain high levels of DOC or TOC, and these may pose a problem in separating Hg from the matrix for stable Hg isotope analysis, as high recovery (>90%) is necessary for sample processing for isotopic analysis to minimize artificial isotopic fractionation. In this study, we found that digesting peatland waters with an acidic mixture of permanganate and persulfate (Balogh et al. 1996) at 95^{o}C for 6-8 hours consistently recovered 90-100% of total Hg from the water sample, thus minimizing the effect of artificial fractionation of Hg isotopes, if any.

From our sample collection, total Hg and MeHg concentration data were variable but similar to previous studies in watershed S2 (Kolka et al. 2001, Mitchell et al. 2008a). There appears to be interannual variation in discharge and thus export of total Hg and MeHg from watershed S2, and there are some positive effects of discharge on total Hg concentration but some negative effects of discharge on MeHg concentration.

Our study is the first to document Hg isotopic compositions in water samples derived from peatland/wetland ecosystem. We found that both MDF and MIF values of Hg in the exported water samples resemble to those of Hg in soil and foliage as reported previously in natural forest ecosystems in Wisconsin (Demers et al. 2013) and northern

California (Tsui et al. 2012), or we collectively call them as “watershed”. Therefore, it may imply that there is minimal or no MDF during desorption of Hg from the soil or bog substrate, and there may be just simple mixing of different masses before being exported from watershed S2. Interestingly, MIF signature of Hg is quite similar among different environmental components collected in watershed S2 and it appears that there is no MIF caused by any processes “within” the watershed, which corroborate the current paradigm that photochemical reactions exclusively cause MIF of stable Hg isotopes in various environmental processes (Bergquist & Blum 2007). Thus, we suggest that MIF signatures can be used as a conservative signature of Hg from the peatland ecosystem for tracing any photochemical changes for Hg after being exported from the watershed.

While the triple mixing model could be useful in identifying sources of Hg contributing to the exported waters, our findings suggest that the majority of Hg exported from watershed S2 is derived from Hg sources “already sequestered” in the watershed itself. Our fine-scale analysis showed that upland and peatland sources have distinct isotopic signatures of Hg (mainly in MDF) and isotopic compositions of Hg in exported waters are found to depend on the hydrology of the watershed (Figure 17).

These results are interpreted in the context of the water retention time in the bog/peatland in watershed S2, and we present a series of schematic diagrams to illustrate the findings (Figure 18). During normal flow, there appears to be a mixing and similar contribution of upland and peatland sources to Hg in the exported waters, and thus MDF values of Hg in the exported waters appear to be between the two endmembers (Figure 18A). During high flow events retention time is decreased and Hg in the exported waters

resembles to the upland sources (Figure 18B). During low to no flow such as summer drought, it would allow longer Hg retention time of water in the peatland (bog) and thus MDF values of Hg in the exported waters resembles to the peatland sources (Figure 18C).

These findings suggest that the changes of climate or weather patterns in the region could have important influences on the hydrology which can mediate Hg cycling within the upland/peatland watershed, and can affect the release of Hg from accumulated peat in the bog. For example, more Hg would be released from the bog if there are more frequent, small-scale drought events while heavy rainfall would release (or export) more Hg from the upland systems.

In this study, we demonstrate the use of an acidic mixture of permanganate and persulfate (Balogh et al. 1996) can release high percentage of Hg from organic matter rich peatland waters, and that can facilitate high-precision analysis of stable Hg isotope ratios via CV-MC-ICP-MS (Blum & Bergquist, 2007). We believe that this analytical approach can provide an important direction for future studies examining complex Hg cycling in peatland and wetland ecosystems, which are often hotspots of Hg cycling and MeHg production. In our study, we successfully show that the use of stable Hg isotopes can provide additional information on source contributions to Hg at a fine scale, which can be combined with information from Hg concentration analyses and/or mass-balance approach (Kolka et al. 2001, Mitchell et al. 2008a), to better understand complex Hg cycling in natural ecosystems.

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APPENDIX A

TABLES

Table 1. Aqueous Samples Collected from MEF: Total Mercury (THg $_{(ng/L)}$) and Methylmercury (MeHg $_{(ng/L)}$) Concentrations.

Sample collection site	Date collected	THg concentration (ng/L)	MeHg concentration (ng/L)
S2 weir	4/9/2014	10.15	0.0306
S2 weir	4/15/2014	16.67	0.0848
S2 weir	4/23/2014	15.47	0.0252
S2 weir	5/13/2014	18.58	0.0957
S2 weir	5/27/2014	13.68	0.2715
S2 weir	6/9/2014	12.12	0.1454
S2 weir	6/23/2014	14.75	0.1611
S2 weir	7/7/2014	14.94	0.1897
S2 weir	7/21/2014	13.36	1.4686
S2 weir	9/16/2014	8.63	0.3844
S2 weir	9/21/2014	12.48	0.3844
S2 weir	10/14/2014	7.84	0.4807
S2 weir	4/12/2015	7.55	0.0644
S2 weir	4/28/2015	4.63	0.1642
S2 weir	5/12/2015	9.56	0.0252
S2 weir	5/26/2015	15.52	0.0553
S2 weir	6/8/2015	16.84	0.0928

S2 weir	6/22/2015	10.93	0.3192
S2 weir	9/7/2015	24.96	0.1235
S2 weir	9/21/2015	10.57	0.0097
S2 weir	10/26/2015	10.50	0.0833
S2 weir	11/9/2015	5.04	0.0653
S2 weir	11/18/2015	21.33	0.1025
S2 weir	11/30/2015	12.76	0.1756
S2 subsurface	4/23/2014	16.91	
S2 subsurface	6/3/2015	23.04	0.0716
S2 subsurface	11/18/2015	23.92	0.08775
S2 surface	4/12/2015	56.82	0.0644
S2 surface	5/12/2015	18.26	0.0252
S2 lagg	4/12/2015	13.10	0.2689
S2 lagg	5/12/2015	10.35	0.3309

Table 2. Solid Matrix Samples Collected from MEF Total Hg Concentrations (ng/g).

Sample collection site	Sample name	THg concentration (ng/g)
Bog core	BC1	83.89
Bog core	BC2	132.96
Bog core	BC3	101.83
Bog core	BC4	92.11
Bog core	BC5	98.05
Bog core	BC6	42.80
Bog core	BC7	34.20
Bog core	BC8	39.93
Bog core	BC8	39.93
Bog core	BC9	93.62
Bog core	BC10	39.64
Vegetation	VG1	18.73
Vegetation	VG4	15.74

Table 3. Previous Published and Unpublished Hg Stable Isotope Data in Different Environmental Compartments.

Sample Type	Sample Location	Sample ID	δ^{202}Hg	σ	Δ^{199}Hg	Δ^{201}Hg	*Data source*
			(‰)	(‰)	(‰)	(‰)	
Precipitation	Wisconsin		-0.74	-1.14	0.5	0.45	*Demers et al., 2013*
Precipitation	Wisconsin		-0.39	-0.79	0.82	0.61	*Demers et al., 2013*
Precipitation	Wisconsin		0.06	-0.34	0.64	0.4	*Demers et al., 2013*
Precipitation	Wisconsin		-0.41	-0.81	0.28	0.5	*Demers et al., 2013*
Precipitation	Wisconsin		-0.23	-0.63	0.16	0.26	*Demers et al., 2013*
Precipitation	Great lakes		-0.39	-0.79	0.5	0.3	*Gratz et al., 2010*
Precipitation	Great lakes		-0.34	-0.74	0.45	0.47	*Gratz et al., 2010*
Precipitation	Great lakes		-0.04	-0.44	0.48	0.05	*Gratz et al., 2010*
Precipitation	Great lakes		-0.29	-0.69	0.22	0.17	*Gratz et al., 2010*
Precipitation	Great lakes		-0.53	-0.93	0.11	0.31	*Gratz et al., 2010*
Precipitation	Great lakes		-0.49	-0.89	0.14	0.37	*Gratz et al., 2010*
Precipitation	Great lakes		-0.21	-0.61	0.23	0.27	*Gratz et al., 2010*
Precipitation	Great lakes		-0.41	-0.81	0.07	0.09	*Gratz et al., 2010*
Precipitation	Great lakes		0.19	-0.21	0.28	0.18	*Gratz et al., 2010*
Precipitation	Great lakes		-0.31	-0.71	0.25	0.18	*Gratz et al., 2010*
Precipitation	Great lakes		-0.52	-0.92	0.24	0.28	*Gratz et al., 2010*
Precipitation	Great lakes		-0.52	-0.92	0.61	0.55	*Gratz et al., 2010*
Precipitation	Great lakes		0	-0.4	0.37	0.47	*Gratz et al., 2010*

Precipitation	Great lakes		-0.5	-0.9	0.1	0.2	*Gratz et al., 2010*
Precipitation	Great lakes		-0.27	-0.67	0.43	0.43	*Gratz et al., 2010*
Precipitation	Great lakes		-0.38	-0.78	0.36	0.35	*Gratz et al., 2010*
Precipitation	Great lakes		-0.4	-0.8	0.36	0.33	*Gratz et al., 2010*
Precipitation	Great lakes		-0.79	-1.19	0.2	0.42	*Gratz et al., 2010*
Precipitation	Great lakes		-0.4	-0.8	0.14	0.23	*Gratz et al., 2010*
Precipitation	Great lakes		0.21	-0.19	0.37	0.17	*Gratz et al., 2010*
Foliage and leaf litter	California	Senesced foliage	-2.53		-0.37	-0.35	*Tsui et al., ES&T, 2012*
Foliage and leaf litter	California	Decomposed bark	-2.12		-0.36	-0.33	*Tsui et al., ES&T, 2012*
Foliage and leaf litter	Wisconsin	Aspen foliage	-1.09		-0.18	-0.21	*Demers et al. 2013*
Foliage and leaf litter	Wisconsin	Aspen foliage	-0.99		-0.22	-0.23	*Demers et al. 2013*
Foliage and leaf litter	Wisconsin	Aspen foliage	-0.96		-0.18	-0.25	*Demers et al. 2013*
Foliage and leaf litter	Wisconsin	Aspen foliage	-1.5		-0.15	-0.15	*Demers et al. 2013*
Foliage and leaf litter	Wisconsin	Aspen foliage	-1.51		-0.2	-0.2	*Demers et al. 2013*
Foliage and leaf litter	Wisconsin	Aspen foliage	-1.34		-0.16	-0.2	*Demers et al. 2013*
Foliage and leaf litter	Wisconsin	Aspen foliage	-1.36		-0.22	-0.23	*Demers et al. 2013*
Foliage and leaf litter	Minnesota	Bog vegetation	-2.30		-0.30	-0.28	*Woerndle*
Foliage and leaf litter	Minnesota	Pine needles	-2.17		-0.43	-0.43	*Woerndle*
Foliage and leaf litter	Minnesota	Pine needles	-2.28		-0.39	-0.38	*Tsui unpublished*
Foliage and leaf litter	Minnesota	Deciduous	-1.67		-0.16	-0.18	*Tsui unpublished*

Soil	New Hampshire	NH-1	-1.83		-0.4	-0.38	*Biswas et al., 2008 EST*
Soil	New Hampshire	NH-1	-1.87		-0.32	-0.32	*Biswas et al., 2008 EST*
Soil	Washington	WA-2	-1.48		-0.19	-0.15	*Biswas et al., 2008 EST*
Soil	Wyoming	WY-1	-0.96		0.03	0.09	*Biswas et al., 2008 EST*
Soil	Minnesota	Bog core 0-5cm	-2.15		-0.34	-0.44	*Woerndle*
Soil	Minnesota	Bog core 5-10cm	-2.24		-0.36	-0.43	*Woerndle*
Soil	Minnesota	Bog core 15-20	-2.06		-0.31	-0.3	*Woerndle*
Soil	Minnesota	Bog core 30-35cm	-1.46		-0.4	-0.41	*Woerndle*
Soil	Minnesota	Bog core 45-50cm	-1.33		-0.33	-0.38	*Woerndle*
S2 waters	Minnesota	4/9/2014	-1.48		-0.2	-0.22	*Woerndle*
S2 waters	Minnesota	S2 Sfc 4/12/14	-1.37		-0.19	-0.28	*Woerndle*
S2 waters	Minnesota	4/12/14	-1.32		-0.12	-0.24	*Woerndle*
S2 waters	Minnesota	S2 Lag 4/12/14	-1.32		-0.25	-0.34	*Woerndle*
S2 waters	Minnesota	S2 Sub	-1.41		-0.29	-0.2	*Woerndle*
S2 waters	Minnesota	4/15/2014	-1.80		-0.3	-0.32	*Woerndle*
S2 waters	Minnesota	4/23/2014	-1.84		-0.13	-0.27	*Woerndle*
S2 waters	Minnesota	5/13/2014	-1.88		-0.26	-0.29	*Woerndle*
S2 waters	Minnesota	5/27/2015	-1.72		-0.22	-0.28	*Woerndle*
S2 waters	Minnesota	S3 6/9/14	-1.41		-0.38	-0.1	*Woerndle*
S2 waters	Minnesota	S2 Sfc 6/9/14	-1.18		-0.22	-0.33	*Woerndle*
S2 waters	Minnesota	6/9/2014	-1.78		-0.32	-0.39	*Woerndle*
S2 waters	Minnesota	6/23/2014	-2.08		-0.32	-0.29	*Woerndle*
S2 waters	Minnesota	7/7/2014	-1.91		-0.2	-0.32	*Woerndle*
S2 waters	Minnesota	7/21/2014	-1.88		-0.2	-0.24	*Woerndle*

S2 waters	Minnesota	9/16/2014	-1.77		-0.16	-0.25	*Woerndle*
S2 waters	Minnesota	9/21/2014	-1.82		-0.25	-0.36	*Woerndle*
S2 waters	Minnesota	10/14/2014	-1.90		-0.3	-0.22	*Woerndle*
S2 waters	Minnesota	4/9/2015	-1.69		-0.26	-0.26	*Woerndle*
S2 waters	Minnesota	4/15/2015	-1.93		-0.31	-0.31	*Woerndle*
S2 waters	Minnesota	6/9/2015	-1.90		-0.29	-0.36	*Woerndle*
S2 waters	Minnesota	6/23/2015	-1.84		-0.26	-0.34	*Woerndle*
S2 waters	Minnesota	9/16/2015	-1.78		-0.18	-0.15	*Woerndle*
S2 waters	Minnesota	9/21/2015	-2.04		-0.35	-0.38	*Woerndle*
S2 waters	Minnesota	10/14/2015	-1.91		-0.31	-0.38	*Woerndle*
S2 waters	Minnesota	10/26/2015	-1.80		-0.22	-0.35	*Woerndle*
S2 waters	Minnesota	11/9/2015	-2.12		-0.3	-0.27	*Woerndle*
S2 waters	Minnesota	11/18/2015	-1.83		-0.33	-0.36	*Woerndle*
S2 waters	Minnesota	11/30/2015	-1.92		-0.31	-0.33	*Woerndle*
S2 waters	Minnesota	S2Lagg 5/13/2015	-1.71		-0.25	-0.32	*Woerndle*
S2 waters	Minnesota	S2Sub 6/9/2015	-1.48		-0.24	-0.34	*Woerndle*
S2 waters	Minnesota	S2Sub 11/18/15	-1.34		-0.24	-0.25	*Woerndle*
Hg0			0.93		-0.15	-0.06	*Demers et al., 2013*
Hg0			1.23		-0.18	-0.2	*Demers et al., 2013*
Hg0			0.48		-0.21	-0.13	*Demers et al., 2013*
Hg0			0.64		-0.21	-0.14	*Demers et al., 2013*
Hg0			0.83		-0.21	-0.18	*Demers et al., 2013*
Hg0			1.6		-0.25	-0.12	*Demers et al., 2013*
Hg0			0.82		-0.12	-0.19	*Demers et al., 2013*
Hg0			0.74		-0.22	-0.14	*Demers et al., 2013*

Hg0			1.32		-0.24	-0.18	*Demers et al., 2013*
Hg0			0.74		-0.17	-0.13	*Demers et al., 2013*
Hg0			0.6		-0.19	-0.05	*Demers et al., 2013*
Hg0			1.33		-0.18	-0.14	*Demers et al., 2013*
Hg0			0.48		-0.04	-0.12	*Gratz et al., 2010*
Hg0			-0.39		-0.17	-0.06	*Gratz et al., 2010*
Hg0			0.27		-0.06	0	*Gratz et al., 2010*
Hg0			0.41		-0.07	-0.09	*Gratz et al., 2010*
Hg0			0.27		0	-0.02	*Gratz et al., 2010*
Hg0			0.25		-0.1	-0.08	*Gratz et al., 2010*
Hg0			-0.59		0.06	0.03	*Gratz et al., 2010*
Hg0			-0.12		-0.11	-0.14	*Sherman et al., 2010*
Hg0			0.15		-0.22	-0.18	*Sherman et al., 2010*
Hg0	Dexte	DXT-VP-1	0.48		-0.04	-0.12	*Gratz et al., 2010*
Hg0	Dexte	DXT-VP-2	-0.39		-0.17	-0.06	*Gratz et al., 2010*
Hg0	Dexte	DXT-VP-3	0.27		-0.06	0	*Gratz et al., 2010*
Hg0	Dexte	DXT-VP-4	0.41		-0.07	-0.09	*Gratz et al., 2010*
Hg0	Dexte	DXT-VP-5	0.27		0	-0.02	*Gratz et al., 2010*
Hg0	Dexte	DXT-VP-6	0.25		-0.1	-0.08	*Gratz et al., 2010*
Hg0	Chicago	UOC-VP-1	-0.59		0.06	0.03	*Gratz et al., 2010*

APPENDIX B

FIGURES

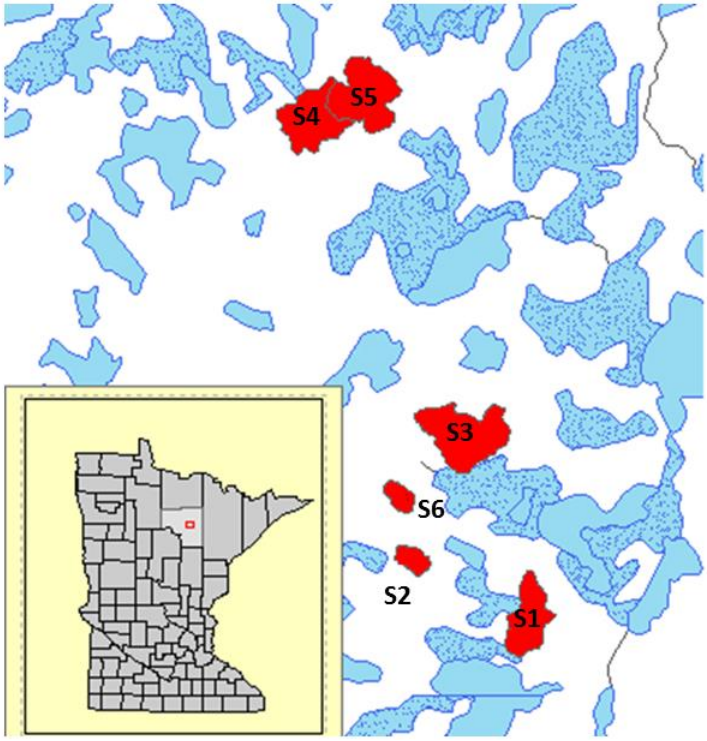


Figure 1. Location of Marcell Experimental Forest (MEF). MEF is located in Itasca County, in the central northern Minnesota. There are six experimental watersheds. Watershed S2 comprising of a peatland and an upland forest is our study site.

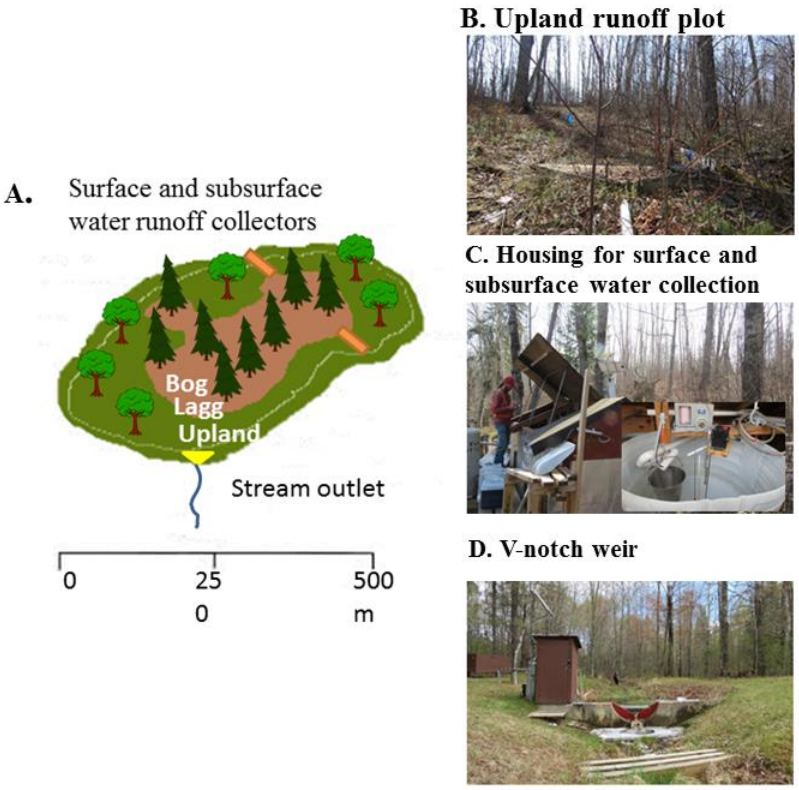


Figure 2. S2 Watershed Features. A. Watershed S2 includes an upland forest, bog, and lagg area and a V-notch weir at the watershed outlet. B. Surface waters were collected from stainless steel troughs through PVC piping on the northern slope of the watershed as well as at a hollow auger well in the lagg (not shown). Subsurface runoff was collected via underground PVC collection pipping (not shown). C. Both surface and subsurface runoff is stored in a housing unit that measures the amount of runoff present before sample collection. D. Watershed S2 export waters were collected from a V-notch weir when flowing. The daily water table level is continuously monitored and recorded on a strip chart in the housing unit near the V-notch weir. Daily precipitation and air temperature are also recorded.

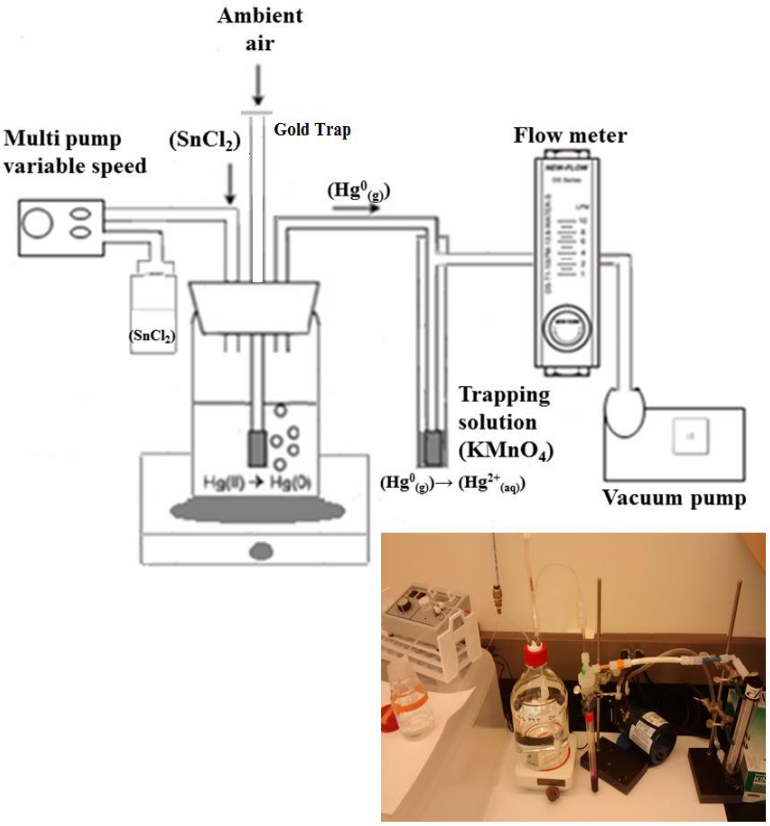


Figure 3. Purge and Trap Method Following Sample Digestion. One liter of digested water samples was placed in a 2 L media bottle with a Corning three-hole delivery cap. 10% $SnCl_2$ was used to continuously reduce $Hg^{2+}_{(aq)}$ into $Hg^{0}_{(g)}$. A vacuum directed the reduced $Hg^{0}_{(g)}$ into a potassium permanganate ($KMnO_4$) solution that oxidizes $Hg^{0}_{(g)}$ to $Hg^{2+}_{(aq)}$, and trapped Hg was analyzed using CVAFS to determine "purge and trap" recovery as well as by CV-MC-ICP-MS for Hg stable isotopic analysis (at the University of Michigan).

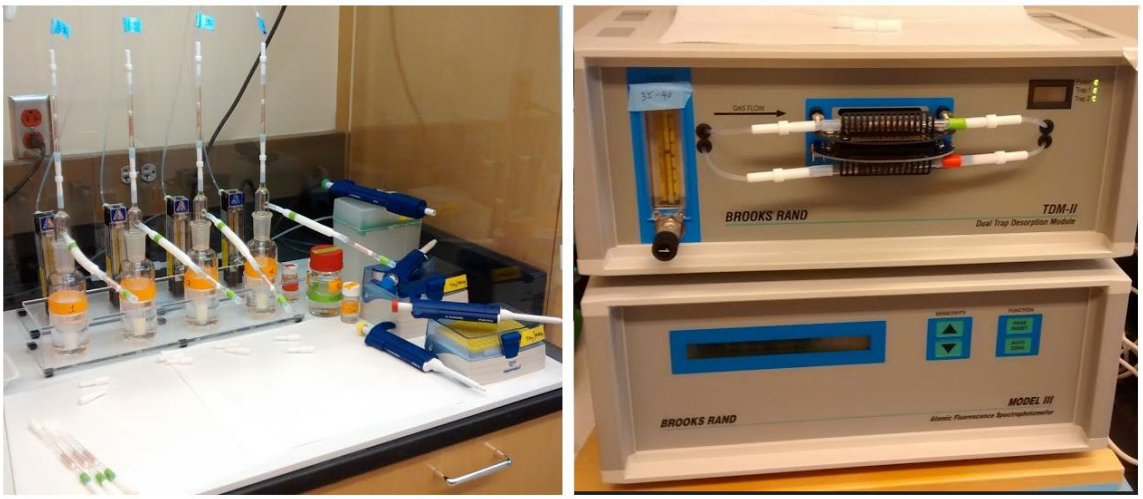


Figure 4. Total Hg Concentration Analysis. Analysis via purging vessels, desorption modules, and CVAFS. Aliquots of acid digested samples were added to purging vessels (pictured left) along with hydroxylamine (NH_2OH) and stannous chloride ($SnCl_2$). Vessels were then purged with Hg-free nitrogen. A gold trap collected Hg purged from the vessels. Gold traps were then run on a Brooks Rand model III CVAFS (pictured right).

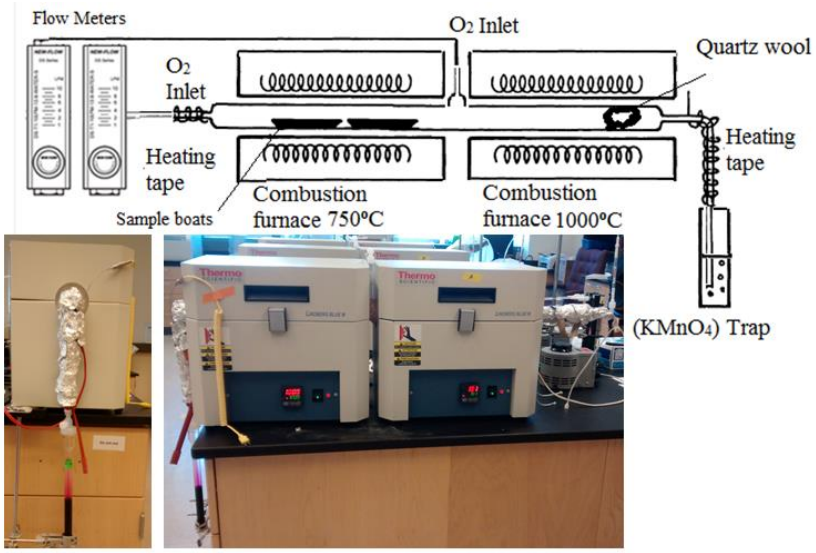


Figure 5. Thermal Separation of Hg from Solid Matrices. The first combustion furnace slowly heated samples to volatilize $Hg^0_{(g)}$ within soil and vegetation samples. $Hg^0_{(g)}$ from the sample was then directed by a stream of Hg-free oxygen into a 1% $KMnO_4$ sample trap. The second furnace was held at 1000°C and contained quartz wool to decompose combustion by-products.

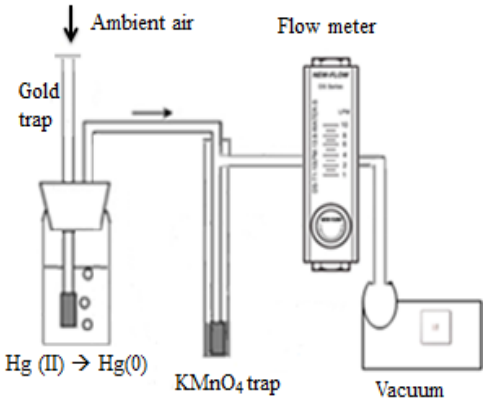


Figure 6. Concentration of Sample Hg Via Purge and Trap**.** Following combustion Hg separated from solid samples were concentrated from a 24 g initial sample trap to a 6 g final sample trap using the purge and trap method. Samples were neutralized with (NH_2OH) and reduced with ($SnCl_2$). A vacuum pump pulled ambient air filtered through a gold trap through to direct $Hg^0_{(g)}$ from the solution into a smaller trap. The contents of the small trap would be eventually analyzed for stable Hg isotopes.

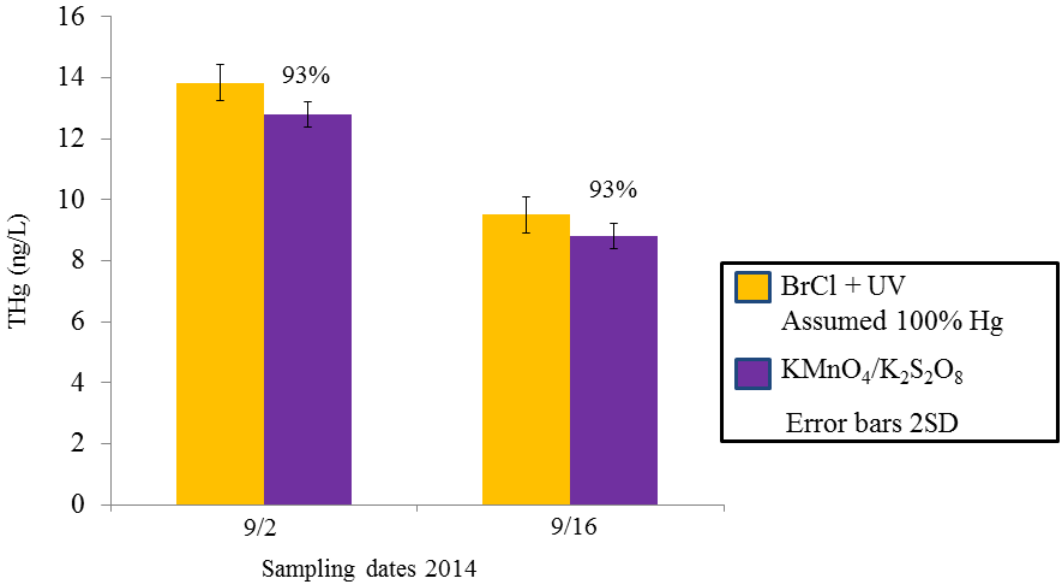


Figure 7. Digestion Method Comparison. Two water samples collected from the watershed S2 on 9/2/14 and 9/16/14 were digested using two different methods (i.e., "BrCl + UV" *vs.* "$KMnO_4/K_2S_2O_8$"). After digestion, each sample was analyzed for total Hg concentration by CVAFS. The permanganate/persulfate digestion recovered 93% of the total Hg as estimated by the digestion with both BrCl and UV treatment which we assumed to release 100% of Hg from all organic matter pools in the water samples. Samples were run in replicate. Error bars show 2SD.

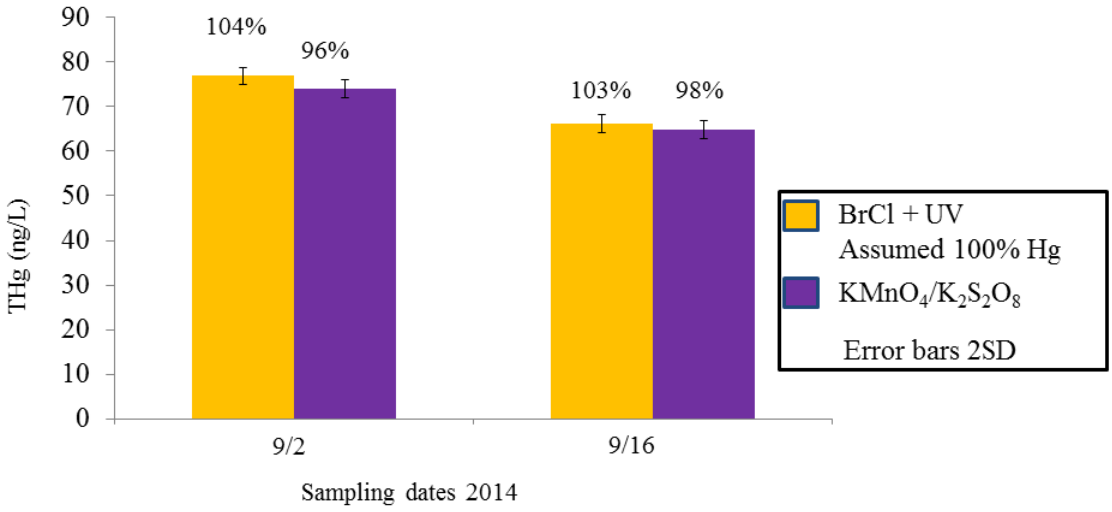


Figure 8. Post Spike Recovery. Two water samples collected from the V-notch weir of S2 on 9/2/14 and 9/16/14 were digested using two different methods. After digestion, each sample were analyzed for total Hg concentration and then spiked with a known amount of Hg standard NIST 3133. These samples were then reanalyzed for Hg concentration, the permanganate digestion recovered 96-98% whereas the BrCl and UV recovered 103-104%. Samples were run in replicate. Error bars show 2SD.

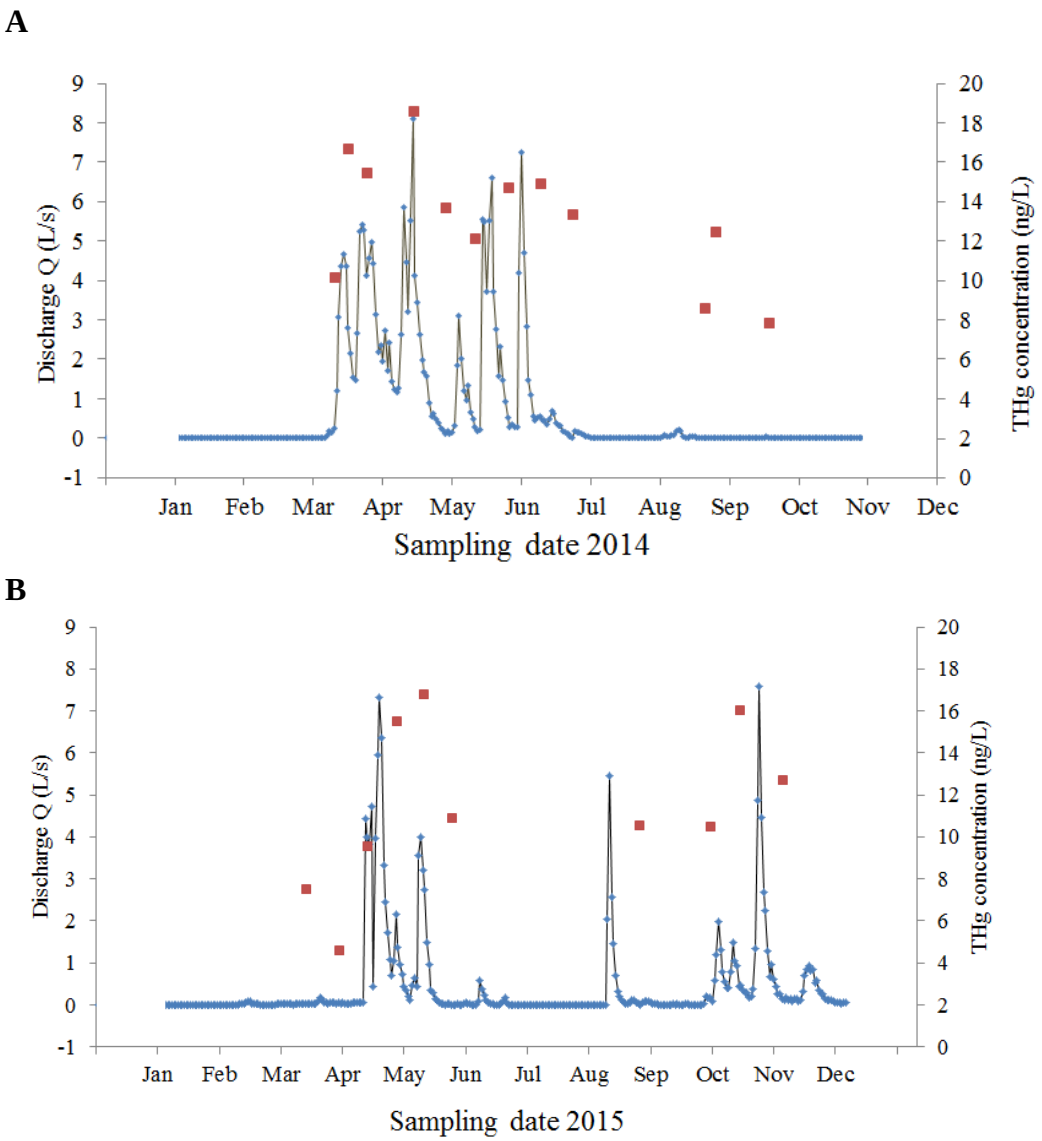
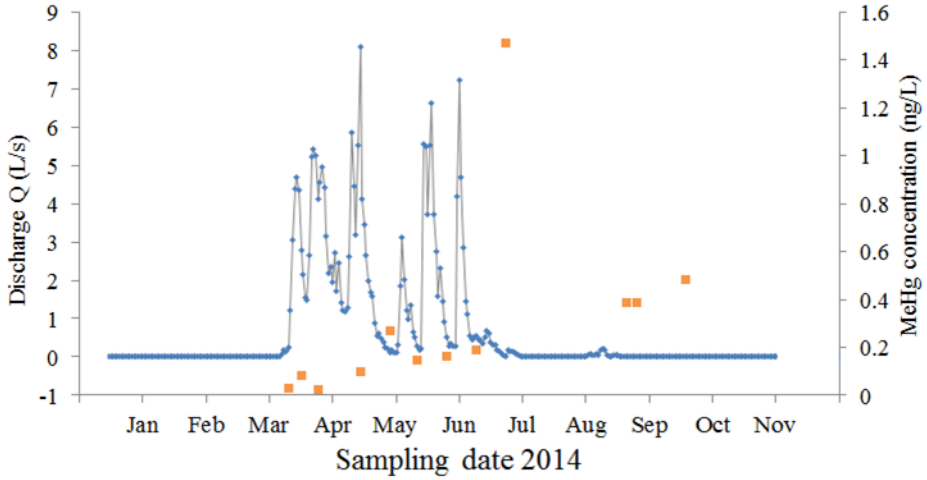


Figure 9. Daily Discharge and Total Hg Concentrations 2014 and 2015. A. Daily discharge Q and total Hg concentrations in 2014. B. Daily discharge Q and total Hg concentrations in 2015. Daily discharge was recorded at watershed S2 V-notch weir. Total Hg concentrations were determined after sample digestion with an acidic mixture of permanganate and persulfate.

A



B

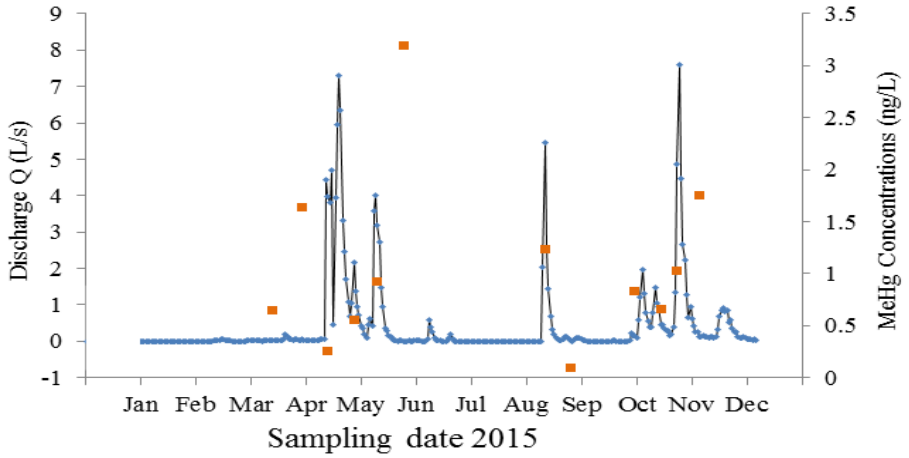
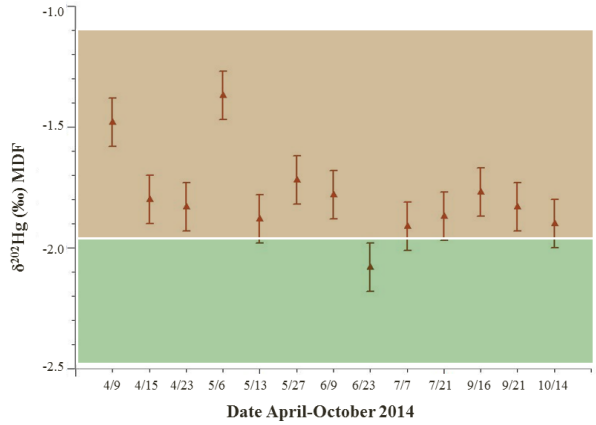


Figure 10. Daily Discharge and MeHg Concentrations 2014 and 2015. A. Daily discharge Q and MeHg concentrations in 2014. B. Daily discharge Q and MeHg concentrations in 2015. Daily discharge was recorded at watershed S2 V-notch weir. MeHg concentrations were determined after distillation and ethylation of samples, and analyzed by pyrolysis-GC CVAFS.

A



B

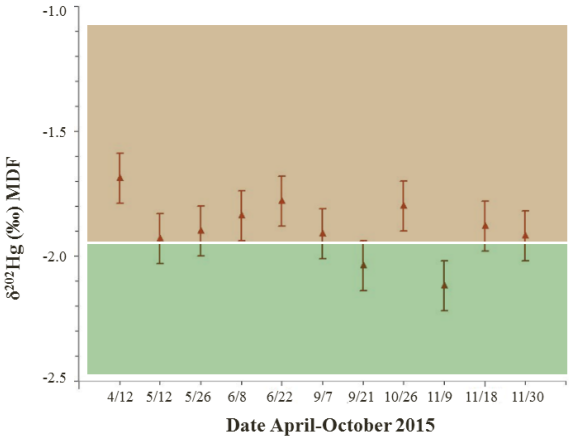
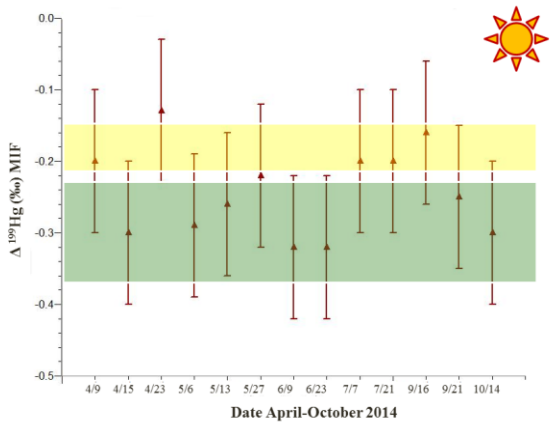


Figure 11. MDF 2014 and 2015. A. Mass-dependent fractionation (MDF) of Hg in the exported waters of from watershed S2 in 2014. B. Mass-dependent fractionation (MDF) of Hg in the exported waters of from watershed S2 in 2015. The brown shaded bar indicates the range of stable Hg isotope values that have been reported for soil (Biswas et al. 2008, Demers et al. 2013, Estrade et al. 2011, Tsui et al. 2012). The green shaded bar indicates the range of stable Hg isotope values reported for vegetation (Demers et al. 2013, Tsui et al. 2012). Error bars (2SD) represent the external analytical reproducibility.

A



B

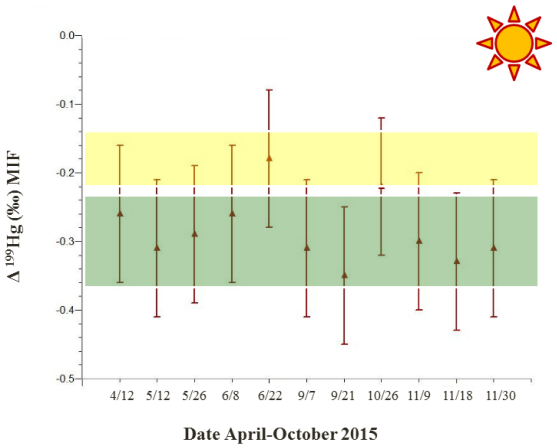
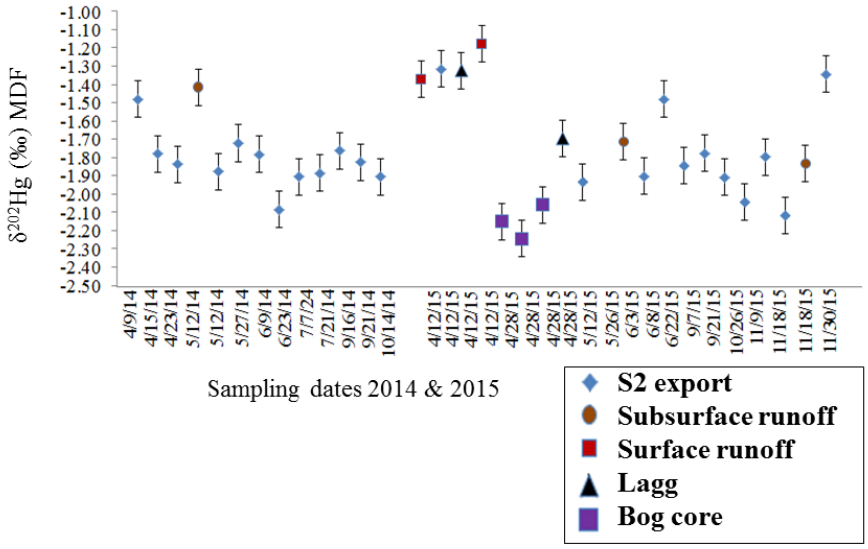


Figure 12. MIF 2014 and 2015. A. Mass-independent fractionation (MIF) of Hg in the exported waters of from watershed S2 in 2014. B. Mass-independent fractionation (MIF) of Hg in the exported waters of from watershed S2 in 2015. Reported MIF range of atmospheric $Hg^0_{(g)}$ is shown in yellow shaded bar (Gratz et al. 2010, Sherman et al. 2012, Demers et al. 2013). Reported MIF range for foliage is shown in green shaded bar (Tsui et al. 2012, Demers et al. 2013). Error bars (2SD) represent the external analytical reproducibility.

A



B

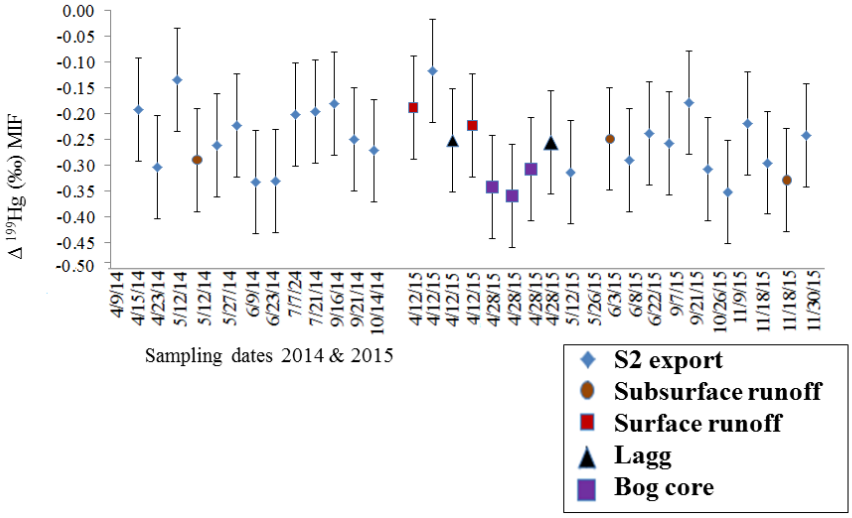


Figure 13. MDF and MIF 2014 and 2015. A. MDF values of Hg in all samples collected from watershed S2 from both 2014 and 2015. B. MIF values of Hg in all samples collected from watershed S2 from both 2014 and 2015. Error bars show the analytical reproducibility at 2SD.

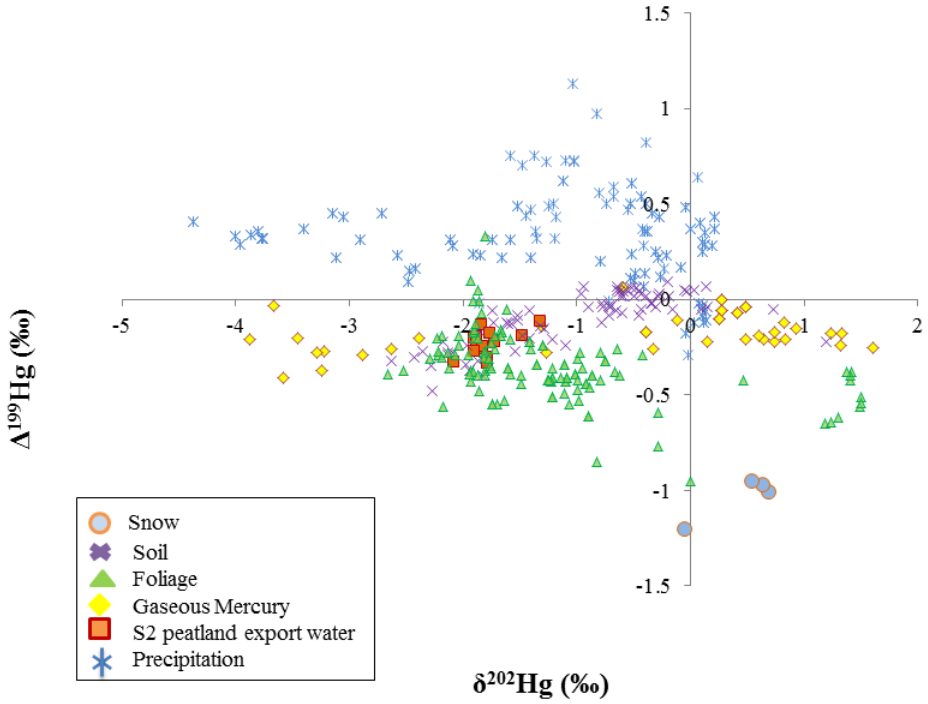


Figure 14. Literature Review of Hg Sources. Possible Endmembers for Sources of Hg Exported from the S2 Watershed. Compared to the S2 export waters collected and analyzed for Hg stable isotopes in 2014 and 2015 shown in orange. Data were obtained from Donavon et al. 2013, Gratz et al. 2012, Chen et al. 2011, Sherman et al. 2012, Gehrke et al. 2011, Gratz et al. 2010, Demers et al. 2013, Rolison et al. 2013, Gratz et al. 2010, Sherman et al. 2010.

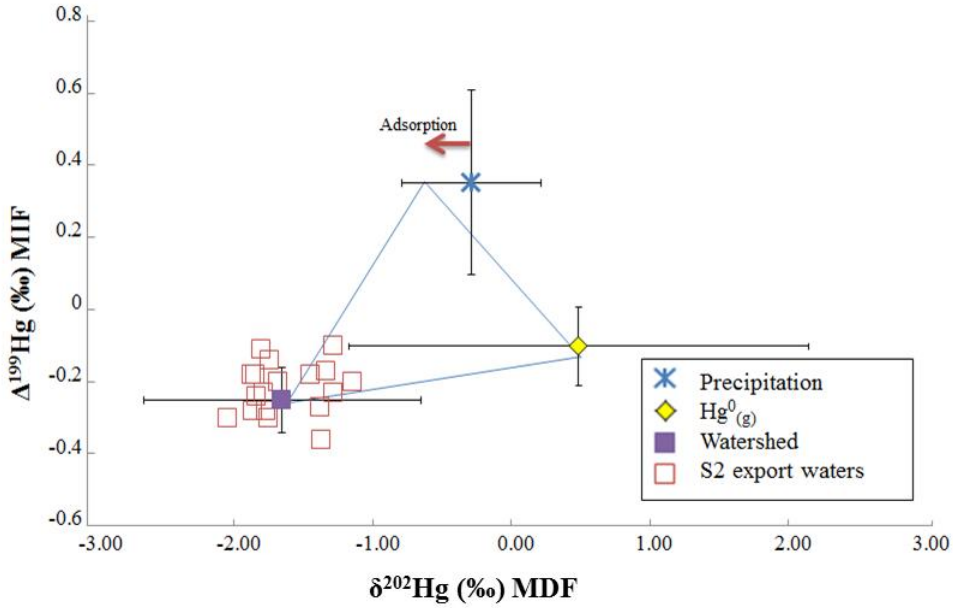


Figure 15. Possible Endmembers for the Triple-Mixing Model. MDF and MIF values of Hg in exported waters from watershed S2 and endmembers used for the triple mixing model. Literature-derived mean isotopic data with one standard deviation error for watershed-derived Hg are from this study and precipitation-derived Hg (n=159) (Donavon et al. 2013, Gratz et al. 2012, Chen et al. 2011, Sherman et al. 2012, Gehrke et al. 2011, Gratz et al. 2010) as well as the associated shift in δ^{202}Hg for adsorption (Jiskra et al. 2012) for precipitation. Gaseous Hg is derived from literature values (n=41) (Demers et al. 2013, Rolison et al. 2013, Gratz et al. 2010, Sherman et al. 2010).

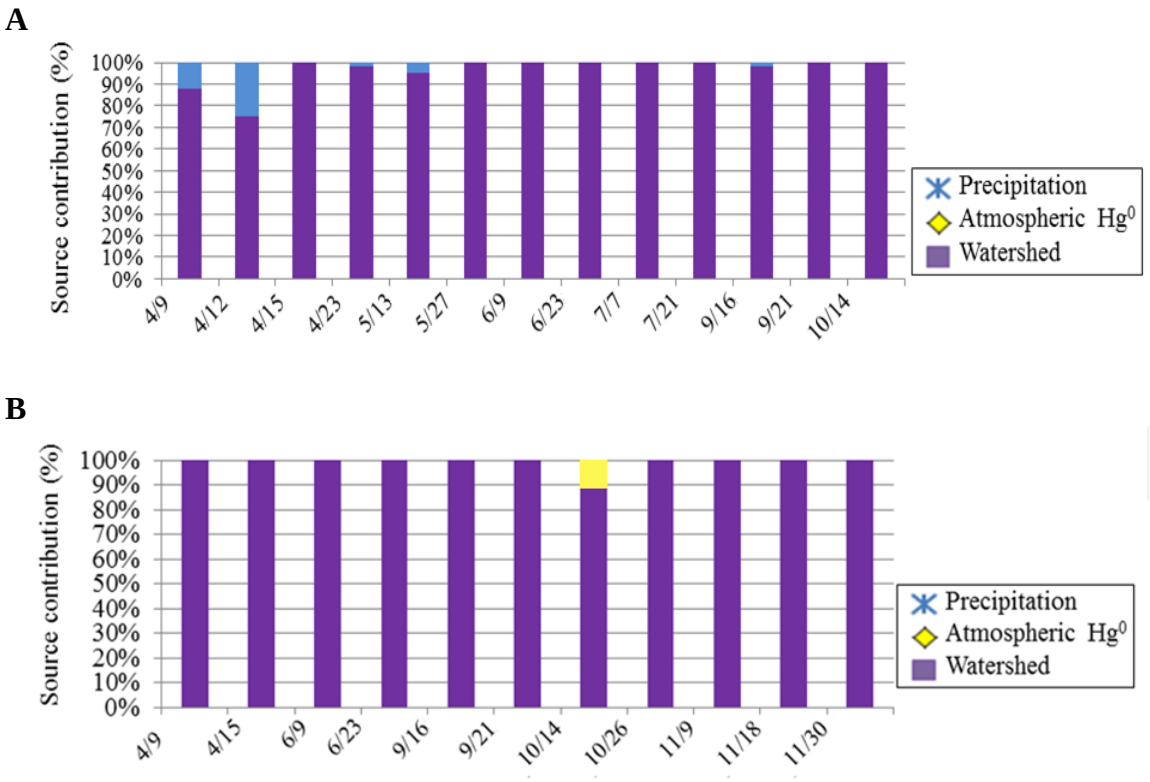


Figure 16. Triple Mixing Model Calculations. A. Source contribution to Hg in exported waters from watershed S2 in 2014. B. Source contribution to Hg in exported waters from watershed S2 in 2015.

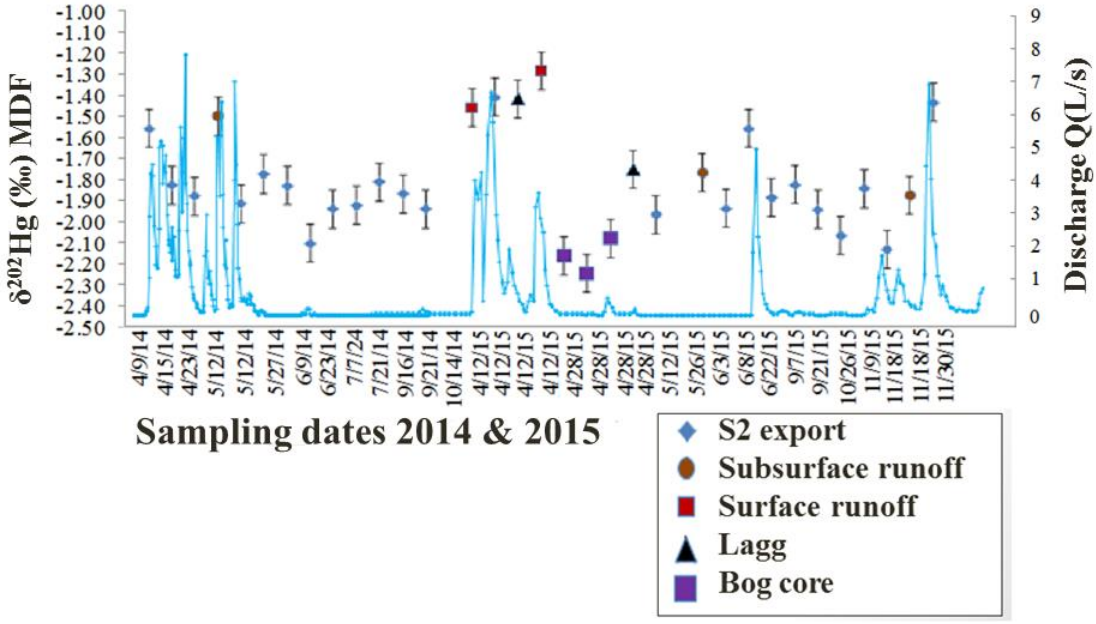
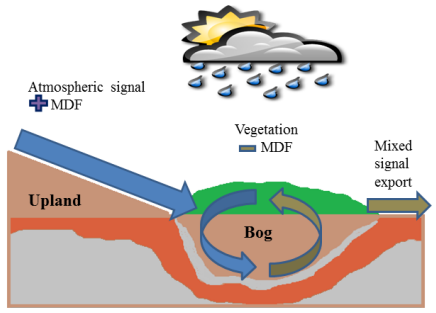
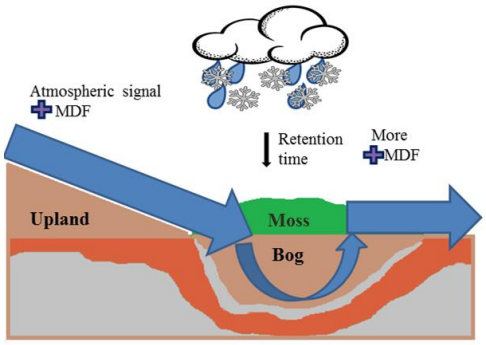


Figure 17. MDF Values of Hg and Discharge 2014 and 2015. Discharge (blue dot and line) for both 2014 and 2015. Samples taken from the v-notch weir are shown in blue diamonds. Bog core includes three samples of bog soil. All samples show an error bar with 2SD for analytical reproducability.

A – Normal flow



B – High flow



C – Low to no flow

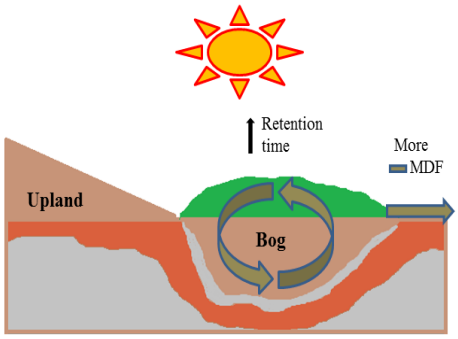


Figure 18. Seasonal Variation of MDF in Watershed S2. Influenced by hydrology in the watershed A. Normal flow. B. High flow. C. Low to no flow.